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Dear Carol,

Here is the material you requested, plus some additional narrative in case you have not received it. If you need additional information please call.

Thanks for your help.

Regards,

Stephen Rothman
Professor of Physiology

P.S. We are not sure why portal vein blood is not substantially higher than systemic blood. We think it means that turnover ~~to the~~ from the circulation is slow for human growth hormone. Mike Berman is preparing nice graphs for much of our data. You might give him a call (476-9262).

Steve Rothman

II. GENE THERAPY BY THE ORAL ROUTE*

A. *SUMMARY*

We propose to utilize epithelial cells of the both the small and large intestines to accomplish systemic gene therapy. The special feature of the method is that it allows for the administration of gene therapy by the oral administration of plasmid/vector containing pills, in amounts and at frequencies found to be efficacious.

B. *INTRODUCTION.*

The intestines provide a large mass of cells (many that normally absorb substances from the gastrointestinal tract into blood), that can be utilized to manufacture gene products of therapeutic interest. Oral administration of desired genes potentially provides the simplest and most widely available means to administer gene therapy.

C. *ADVANTAGES OF THE APPROACH.*

1. Genes are administered by the prescription of "gene pills", avoiding medical procedures, either invasive or non-invasive.

2. It allows for the convenient application of gene therapy by the oral administration of plasmid/vector formulations at frequencies found to be efficacious.

3. The substantial mass of epithelial cells lining the small and large intestines can be recruited to produce large quantities of a chosen gene product, or group of products. Most of these cells normally function to transport substances from the intestinal lumen into the bloodstream.

4. Intestinal function is under nervous and hormonal control, and thus it may also be possible to modulate the release of product through physiological and pharmacological mechanisms.

5. The method provides the means for the simple, cost efficient, and repeated administration of a chosen gene, with the minimal danger of serious side effects.

6. The method can be used for the treatment of autoimmune diseases.

6. Finally, transfection can be used to treat diseases of the gastrointestinal tract itself.

D. *GENE THERAPY BY PILL.*

Gene therapy through the gut requires no special medical facilities and can be administered by the simple prescription of a drug. It can be used to: 1) cure genetic diseases; 2) provide therapy for protein and hormone deficiencies; and 3) serve as an

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* The date of this invention has been documented in a letter to Linda Carloni of the Office of Technology Transfer of the University of California, and will be the subject of a patent application by the University of California.

adjunctive treatment with other forms of therapy. The array of disease processes that can be helped by such treatments, and the number of proteins whose production could be affected to provide such treatments is substantial (see appended tables).

E. *THE PRINCIPAL INVESTIGATORS*

Three UCSF professors have been involved in this project. Ira Goldfine, Professor of Physiology and Medicine; Michael German, Assistant Professor of Medicine; and Stephen Rothman, Professor of Physiology and Stomatology. Each brings different expertise to this common endeavor. Dr. Rothman is an expert on exocrine protein secretion and the gastrointestinal system, Dr. Goldfine is an expert in diabetes and metabolic disorders, and Dr. German is an expert on gene expression through genetic transformation. Together, they provide the range of expertise necessary to achieve all the experimental aims that have been set out.

F. *PRELIMINARY RESULTS.*

We have recently obtained our first results with gut therapy. Different gene and vector formulations for HGH have been administered to segments of intestine of approximately 5 cm length. Elevations in HGH levels in blood some 4 times that of controls have been achieved after the administration of the plasmid to the upper duodenum and terminal ileum (figure 6). Animals were studied at both 24 and 48 hours after transformation. Plasma levels were similarly elevated after both duodenal and ileal administration.

G. *PROJECT PLAN.*

Phase 1

1. Experimental design and goals.

1. *Studies in anesthetized rats.* Segments of small and large bowel will be isolated and the efficacy of transfection assessed as discussed in the description of the project plan for secretory gland expression. The plasmid/vector formulation will be injected into the bowel for various periods and at various volumes to access effectiveness. Subsequently, blood levels of products will be measured, and transformed tissue extracted and assayed for the presence of the engineered product.

2. *Studies in awake rats.* In this case, instillation of the product into the duodenum of awake rats will be the mode of plasmid/vector administration. The amount and duration of administration will be varied and levels in blood followed over time.

2. A brief summary of a some methods to be employed.

<u>Animal preparations.</u>

1. In animals anesthetized with pentobarbital, various areas of the intestines will be exposed and isolated. Segments of about 5 cm in length will be used initially, and the plasmid/vector formulation injected into the isolated segment for various periods of time in various volumes. Subsequent to administration of the plasmid/vector, the abdomen is closed and the animal allowed to recover.

2. For studies in awake animals, a catheter is installed into the upper duodenum, exiting the body in the rear of the neck. Material will be injected directly into the upper small bowel through this catheter.

Assays:

As noted above for the secretory gland work.